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Recognition of the acceptor β-D-Glc*p*NAc-(1 → 2)-α-D-Man*p*-(1 → 6)-β-D-Glc*p*-OR by *N*-acetylglucosaminyltransferase-V: None of the hydroxyl groups on the Glc-residue are important

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ABSTRACT

The enzyme, *N*-acetylglucosaminyltransferase-V (GlcNAcT-V, E.C 2.4.1.155), transfers a β-D-Glc*p*NAc residue, from UDP-GlcNAc, to the OH-6 group of the Man residue in the synthetic acceptor β-D-Glc*p*NAc-(1 → 2)-α-D-Man*p*-(1 → 6)-β-D-Glc*p*-O$(CH_2)_7CH_3$ (**3**). Trisaccharide **3** is an excellent substrate for the enzyme from hamster kidney with a K_m value of 26 μM. In this paper we examine the contribution of the Glc residue in **3** to acceptor recognition by this enzyme. β-D-Glc*p*NAc-(1 → 2)-α-D-Man*p*-O$(CH_2)_7CH_3$ (**5**), where the Glc residue in **3** has been deleted, was synthesized and found to be a very poor substrate with a K_m value elevated to almost 2 mM. Two other analogues of **3**, where the Glc residue was *O*-trimethylated (**6**) or *O*-tribenzylated (**7**), respectively, possessed K_m values very near to those of **3**. The Glc residue in **3** is thereby shown to present an important recognition element for GlcNAcT-V, but none of the free hydroxyl groups are required. This observation should facilitate the design of more hydrophobic and membrane-permeable analogues of **3** that are expected to function as specific glycosylation inhibitors.

INTRODUCTION

The branching pattern of asparagine-linked oligosaccharides is controlled by a series of *N*-acetylglucosaminyltransferases (GlcNAcT's) numbered I–VI[1,2]. Of these enzymes, GlcNAcT-V[3] has taken a position of prominence in glycobiology since the activity of this enzyme was shown to correlate with the metastatic potential of several tumor cell lines[4,5]. GlcNAcT-V biosynthetically converts glycopeptide acceptors with the minimum structure **1** to the β-(1 → 6) branched tri- or tetra-antennary structure(s) **2**. Efforts to understand the biochemical events that regulate the expression of GlcNAcT-V in tumor cells are in progress[5,6]. The enzyme has also become a major target for the design of glycosyltransferase inhibitors that might have potential for reversing the metastatic phenotype[6–8].

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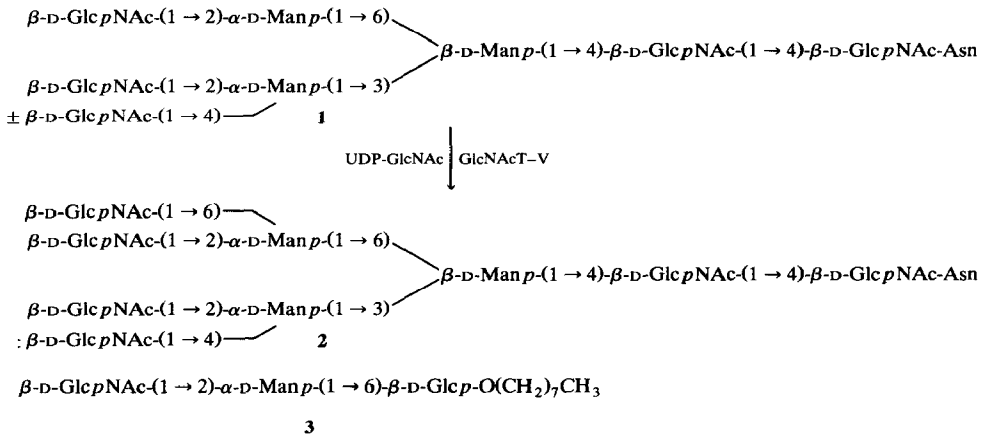
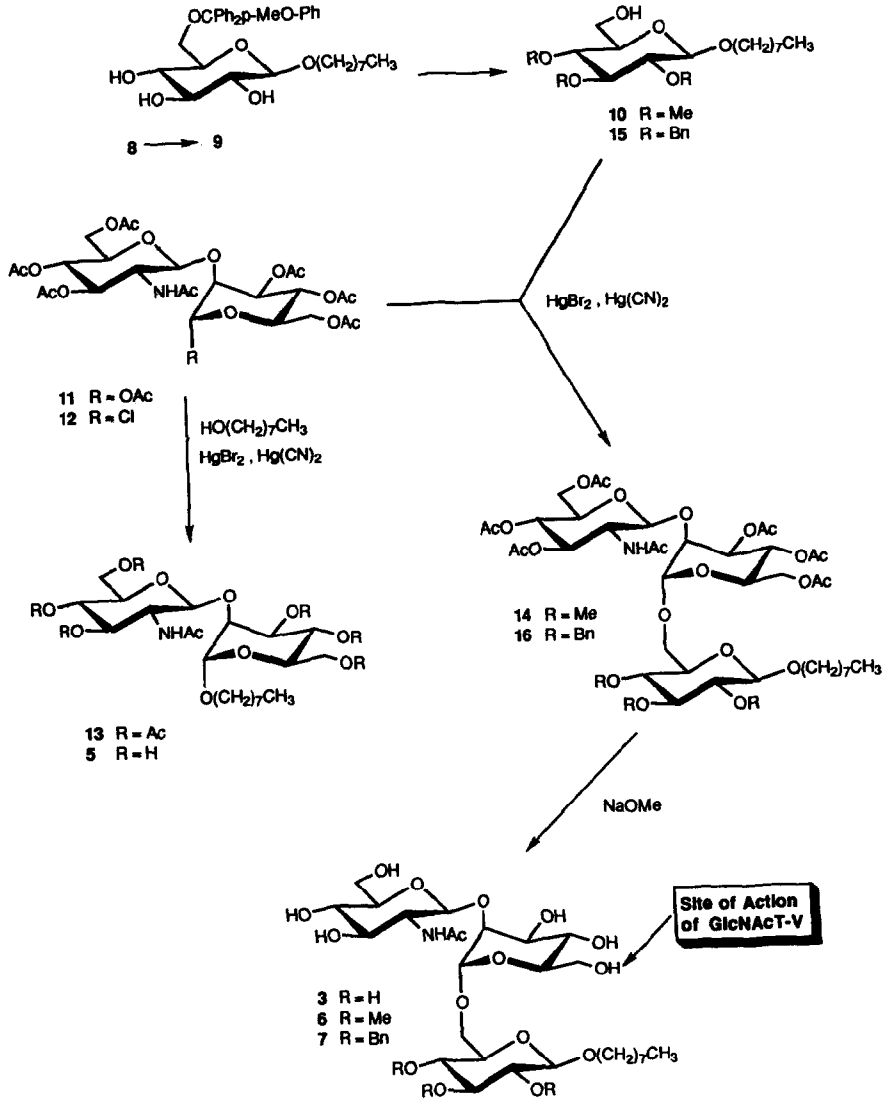
Oligosaccharides much simpler than **1** have been shown to also act as acceptors for GlcNAcT-V and have been extensively used to assay the activity of this enzyme[9–11]. The simplest and most readily synthesized of these is the trisaccharide **3** (ref 10) where the chitobiosyl–Asn grouping has been replaced by a hydrophobic octyl moiety and the oligosaccharide linked α-(1 → 3) to the branched β-D-Man*p* residue has been removed. In addition, the 'core' β-D-Man*p* residue that is common to all Asn-linked oligosaccharides has been replaced by the more chemically accessible β-D-Glc*p* unit. Removal of the OH-6″ group (of the α-D-Man*p* residue) in **3** (the OH group to which GlcNAcT-V transfers a β-D-Glc*p*NAc residue) produces the deoxy-trisaccharide β-D-Glc*p*NAc-(1 → 2)-α-D-[6-deoxy-Man*p*]-(1 → 6)-β-D-Glc*p* -O$(CH_2)_7CH_3$ (**4**), which has been shown[6] to be an inhibitor (K_i near 30 μM) for GlcNAcT-V. This compound, however, appears[12] to penetrate cells very poorly and is therefore not a useful reagent for modification of cell-surface glycosylation in living cells. The poor membrane permeability of **4** is undoubtedly due to the hydrophilic nature of this trisaccharide as a consequence of the large number of OH groups present on the molecule.

In an effort to produce more hydrophobic derivatives of **4**, we decided to examine which of the OH groups in acceptor **3** (and therefore, by inference, in inhibitor **4**) were important for recognition by GlcNAcT-V. We report here the synthesis of disaccharide **5**, where the entire Glc residue has been deleted and replaced by an octyl aglycone, and the trisaccharides **6** and **7** where the OH groups on the Glc residue have been *O*-methylated and *O*-benzylated, respectively. These three new compounds were then kinetically evaluated as acceptor substrates for GlcNAcT-V.



RESULTS AND DISCUSSION

Protection of the primary hydroxyl group in octyl β-D-glucopyranoside (**8**) (See Scheme 1) as its mono-*p*-methoxytrityl ether (**9**) (ref 10) allowed its tri-*O*-methyl-



Scheme 1.

ation and, after removal of the trityl ether protecting group, yielded alcohol **10** (78%). The required β-D-Glc*p*NAc-(1 → 2)-α-D-Man*p* donor **12** was then prepared from the known[13] peracetylated disaccharide **11**. In previous work[13], the labile glycosyl bromide of **11** was prepared for this purpose. We found it more convenient, however, to prepare the chloride **12**, which proved to be a stable crystalline compound. Reaction of **12** with octanol under Helferich conditions afforded the α-linked disaccharide derivative **13** (58%), while glycosylation of **10** under the same conditions afforded trisaccharide **14** (65%). Glycosylation of the

previously reported[10] tri-*O*-benzyl derivative **15** proceeded also to give the α-linked trisaccharide **16** (51%). Removal of the acetyl protecting groups in **13**, **14**, and **16**, using sodium methoxide in methanol, then furnished the target compounds **5–7**.

Compounds **5–7** were tested as acceptors for GlcNAcT-V that was partially purified from hamster kidney according to published procedures[7]. The enzyme assay quantitated the transfer of radiolabelled GlcNAc from UDP-[^{3}H]GlcNAc to the acceptors. To facilitate the separation of the radiolabelled products from unreacted UDP-[^{3}H]GlcNAc and its breakdown products, all three potential acceptors were synthesized attached to the octyl aglycone. This rendered them sufficiently hydrophobic to be quantitatively adsorbed onto C_{18} cartridges[11]. Elution of the product with methanol, followed by liquid scintillation counting, then permitted the quantitation of enzymatically formed product. It is assumed that the glycosylation proceeds normally with these new substrates, with transfer of GlcNAc to OH-6 of the α-D-Man*p* residue in the acceptors, though this was not rigorously proven in the present study. The product of GlcNAcT-V on trisaccharide acceptors such as those reported here has been isolated[14] and shown to be the expected 6′-*O*-glycosylated tetrasaccharide. The enzyme assays were also conducted in the presence of EDTA since GlcNAcT-V is the only GlcNAc-transferase acting in the core-region which retains activity in the absence of manganese ions[1,2]. The results of the kinetic evaluations are reported in Table I.

The K_m for the parent trisaccharide **3** was 26 μM, in accord with values previously determined for this compound, with a V_{max} value of 75 pmol/h with the present enzyme preparation under standard assay conditions. Octyl disaccharide **5**, where the Glc residue is absent, is seen to be very poorly recognized by the enzyme since the K_m increases close to two orders of magnitude to near 2 mM. This suggests that the Glc residue provides an important recognition element for GlcNAcT-V.

The tri-*O*-methyl trisaccharide **6** was found to be an excellent substrate, with kinetic parameters almost indistinguishable from those of the parent **3**. The tri-*O*-benzyl compound **7** was not soluble in aqueous buffers, so Me_2SO (10%) had

TABLE I

Kinetic parameters for GlcNAc-T V acceptor analogues

Acceptor	K_m (μM)	V_{max} (pmol/h)	V_{max}/K_m	% relative V_{max}
3	26.4 ± 1.7	75.2 ± 1.4	2.8	100 [a]
5	1843 ± 353	102 ± 7	0.055	137 [a]
6	33.1 ± 2	109 ± 2	3.3	145 [a]
3 + Me_2SO	36.9 ± 0.6	150 ± 8	4.1	100 [b]
7 + Me_2SO	58 ± 5	26 ± 1	0.45	17 [b]

[a] Rate relative to that obtained using **3** as acceptor under standard conditions. [b] Rate relative to that obtained using **3** as acceptor in the presence of 10% Me_2SO as described in the Experimental section.

to be added to the assay mixtures to overcome this difficulty. In the presence of Me_2SO, the K_m for the reference standard **3** increased slightly to 37 μM, while the transfer reaction proceeded faster as reflected in a 100% increase in V_{max} to 150 pmol/h. Using these latter values as a basis for comparing the substrates in aqueous Me_2SO, tri-*O*-benzylation of the glucose residue in **3**, to produce **7**, can be seen to result in only a slight (near 50%) increase in the K_m to 58 μM. The V_{max} for this compound, unlike for **5** and **6**, was considerably lower. Formation of the enzyme–substrate complex is therefore seen to be only marginally affected by what amounts to very large perturbations of the Glc residue. These perturbations block all of the hydroxyl groups and dramatically increase the size and potential steric interactions of the acceptor analogs with the enzyme combining site.

The remarkable results described above indicate that none of the OH groups on the Glc residue are important for recognition and binding to the enzyme, even though removal of the Glc residue very detrimentally reduced acceptor activity. This result suggests a specific hydrophobic recognition of the Glc residue and its aglycone by GlcNAcT-V. It is hoped that other hydrophobic groups may be able to replace the role of the Glc residue in binding to the enzyme, thereby providing easy access to more membrane-permeable GlcNAcT-V acceptors and inhibitors.

EXPERIMENTAL

General methods.—TLC was performed on Silica Gel 60-F_{254} (E. Merck) with detection by quenching of fluorescence and by charring with H_2SO_4. Column chromatography was performed on Silica Gel 60 (E. Merck, 40–63 mm). The following solvent systems were used: *A*, hexane–EtOAc 4:1; hexane–EtOAc–MeOH mixtures *B*, 6:3:1; *C*, 6:4:1; *D*, 8:2:1; *E*, 8:4:1; *F*, 6:2:1; *G*, CH_2Cl_2–MeOH 4:1. Optical rotations were measured with a Perkin–Elmer 241 polarimeter at 23°C. Melting points were measured with Fisher–Johns melting point apparatus. ^1H NMR spectra were recorded at 300 MHz (Bruker AM 300) and ^{13}C NMR spectra were obtained at 75.5 MHz on the same instrument. Only partial NMR data are reported, as the other data were in accord with the proposed structures. Fast-atom bombardment spectra (FABMS) were recorded on a Kratos AEI MS9 instrument with Xe as the bombarding gas and 5:1 1,4-dithiothreitol–1,4-dithioerythritol as the matrix. Elemental analyses were carried out on a Carlo Erba EA1108 instrument.

Materials.—Millex-GV (0.22 μm) filter units were from Millipore Corp. (Mississauga, ON). EDTA, Triton X-100, and UDP-GlcNAc were obtained from Sigma Chemical Co. (St. Louis, MO). Hamster kidneys were obtained from Keystone Biologicals (Cleveland, OH). Ecolite(+) liquid scintillation cocktail was from ICN Biomedicals (St. Laurent, P.Q). Reverse-phase C_{18} SepPak cartridges from Waters Associates (Missisuaga, ON) were pre-equilibrated with 20 mL of MeOH and 50 mL of water before use. UDP-[6-^{3}H(N)]GlcNAc was obtained from American Radiolabelled Chemicals (St. Louis, MO). In order to reduce background values

obtained in radioassays, this material was lyophilized, passed through a C_{18} SepPak cartridge pre-equilibrated with water, and then re-lyophilized and dissolved in 7:3 EtOH–water for use. Other materials were of reagent grade.

Octyl 2,3,4-tri-O-methyl-β-D-glucopyranoside (**10**).—Compound **9** (0.50 g, 0.89 mmol) was dissolved in dry DMF (10 mL) and stirred at room temperature for 1 h with NaH hydride (4.17 mmol). After cooling to 0°C, iodomethane (0.26 mL, 4.18 mmol) was added dropwise, and the mixture was stirred for 16 h at room temperature. The mixture was poured into ice–water (40 mL), stirred for 1 h, then extracted with CH_2Cl_2 (3 × 20 mL). Washing with satd aq NH_4Cl, then water, followed by drying and concentration gave 0.70 g of crude product. TLC showed a major spot at R_f 0.53 (solvent *A*). The product was stirred at room temperature for 20 h with 80% aq acetic acid (15 mL) then concentrated to leave a residue that was purified by chromatography (solvent *A*) to give **10** as a syrup (230 mg, 78%): R_f 0.17 (solvent *A*), $[\alpha]_D$ −16.9° (*c* 1.3, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 4.27 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 3.89 (dd, 1 H, $J_{2,3}$ 6.5, $J_{3,4}$ 9.5 Hz, H-3), 3.63, 3.58, 3.55 (each s, 3 H, OCH_3), 3.49 (dd, 1 H, $J_{3,4}$ 9.5, $J_{4,5}$ 6.9 Hz, H-4), 1.99 (br, 1 H, *OH*, D_2O exchangeable), and 0.88 (t, 3 H, *J* 6.5 Hz, CH_3); ^{13}C NMR ($CDCl_3$): δ 103.6 (C-1), 60.8, 60.5, 60.4 (OCH_3), and 14.1 (CH_3). Anal. Calcd for $C_{17}H_{34}O_6$: C, 61.05; H, 10.25. Found: C, 61.34; H, 9.97.

2-O-(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-3,4,6-tri-O-acetyl-α-D-mannopyranosyl chloride (**12**).—Compound **11** (0.81 g, 1.2 mmol) was dissolved in dry CH_2Cl_2 (20 mL), and Ac_2O (1.0 mL, 10.6 mmol) was added under Ar at 0°C. HCl was bubbled through the solution for 20 min, and the mixture was allowed to warm up to room temperature and stirred for 16 h. The solution was poured into ice–water (50 mL) and stirred for 30 min. The organic phase was separated, washed with cold satd $NaHCO_3$, water, dried ($MgSO_4$), and concentrated to yield 0.77 g of crude product. Crystallization (EtOAc–Et_2O) gave pure **12** (0.40 g, 51%). Chromatography of the mother liquor (solvent *B*) yielded more **12** (0.16 g, 20%): R_f 0.28 (solvent *C*); mp 215°C (dec); $[\alpha]_D$ +18.5° (*c* 1.1, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 6.45 (d, 1 H, $J_{NH,2'}$ 8.1 Hz, N*H*), 6.00 (d, 1 H, $J_{1,2}$ 1.2 Hz, H-1), 5.49 (dd, 1 H, $J_{2',3'}$ 10.6, $J_{3',4'}$ 9.5 Hz, H-3′), 5.11 (d, 1 H, $J_{1',2'}$ 8.4 Hz, H-1′), 5.09 (t, 1 H, $J_{3',4'} = J_{4',5'} = 9.5$ Hz, H-4′), 2.12–2.01 (cluster of s, 18 H, 6 × OAc), and 1.96 (s, 3 H, $COCH_3$); ^{13}C NMR ($CDCl_3$): δ 171.1–169.4 (7 × CO), 98.7 (C-1′), 89.5 (C-1), 55.1 (C-2′), 23.1 (*C*OCH3), and 20.7–20.5 (6 × OAc). Anal. Calcd for $C_{26}H_{36}ClNO_{16}$: C, 47.75; H, 5.55; Cl, 5.42, N, 2.14. Found: C, 47.79; H, 5.44; Cl, 5.81; N, 2.18.

General procedure for glycosylations.—To a solution of acceptor alcohol in a 1:1 mixture of benzene–nitromethane (20 mL) was added 4A molecular sieves (200 mg) and mercuric cyanide (88 mg, 0.35 mmol). The mixture was cooled under Ar to 0°C, and a solution of **12** in the same solvent (1.5 mL) was added dropwise. In all cases, no reaction was observed after 3 h at room temperature, at which point mercuric bromide (126 mg, 0.35 mmol) was added. The mixtures were stirred at room temperature for 16 h then heated at 40°C until no more **12** was detected by

TLC. The mixture was filtered through Celite and washed with CH_2Cl_2 (3 × 20 mL). The combined filtrates were washed with satd $NaHCO_3$, M KI, and water. Drying and concentration afforded the crude product which was further purified by chromatography.

Octyl 2-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-3,4,6-tri-O-acetyl-α-D-mannopyranoside (**13**).—Reaction of 1-octanol (39 mg, 0.3 mmol) with **12** (131 mg, 0.20 mmol) as described above left a residue (250 mg) that was purified by chromatography (solvent *D*) to give **13** as a solid (87 mg, 58%): R_f 0.34 (solvent *E*); mp 172–175°C; $[\alpha]_D$ −0.9°(*c* 1 $CHCl_3$). ^1H NMR ($CDCl_3$): δ 5.00 (d, 1 H, $J_{1',2'}$ 8.2 Hz, H-1'), 4.68 (d, 1 H, $J_{1,2}$ 1.2 Hz, H-1), 3.52 (ddd, 1 H, $J_{1',2'}$ 8.2, $J_{2',3'}$ 10.5 Hz, $J_{NH,2'}$ 7.9 Hz, H-2'), 2.09–1.98 (cluster of s, 18 H, 6 × OAc), 1.93 (s, 3 H, $COCH_3$), and 0.88 (t, 3 H, *J* 6.5 Hz, CH_3); ^{13}C NMR ($CDCl_3$): δ 170.9–169.6 (7 × *C*O), 98.7, 97.6 (C-1, C-1'), 55.8 (C-2'), 23.3 ($COCH_3$), 20.8–20.6 (6 × OAc), and 14.1 (CH_3). Anal. Calcd. for $C_{34}H_{53}NO_{17}$: C, 54.61; H, 7.14; N, 1.87. Found: C, 54.82; H, 7.25; N, 1.89.

Octyl 6-O-[2-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-3,4,6-tri-O-acetyl-α-D-mannopyranosyl]-2,3,4-tri-O-methyl-β-D-glucopyranoside (**14**). —Glycosylation of **10** (67 mg, 0.2 mmol) with **12** (164 mg, 0.25 mmol) as described above gave a crude product (235 mg), which was purified by chromatography (solvent *F*) to give unreacted starting alcohol (**10**, 13 mg, 19%, R_f 0.65 in solvent *C*) and **14** (124 mg, 65%) as a syrup; R_f 0.27 (solvent C); $[\alpha]_D$ −8.0° (*c* 1, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 4.97 (d, 1 H, $J_{1'',2''}$ 8.3 Hz, H-1''), 4.79 (d, 1 H, $J_{1',2'}$ 1.4 Hz, H-1'), 4.23 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1), 3.63, 3.58, 3.63 (each s, 3 H, OCH_3), 2.11–2.01 (cluster of s, 18 H, 6 × OAc), 1.93 (s, 3 H, $COCH_3$), and 0.87 (t, 3 H, *J* 6.5 Hz CH_3); ^{13}C NMR ($CDCl_3$): δ 170.7–169.4 (7 × CO), 103.6 (C-1), 98.6 and 97.8 (C-1', C-1''), 60.7 60.5 60.4 (OCH_3), 55.6 (C-2''), 23.3 ($COCH_3$), 20.7–20.6 (6 × OAc), and 14.1 (CH_3). Anal. Calcd for $C_{43}H_{69}NO_{22}$: C, 54.25; H, 7.31; N, 1.47. Found: C, 54.41; H, 7.40; N, 1.52.

Octyl 6-O-[2-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-3,4,6-tri-O-acetyl-α-D-mannopyranosyl]-2,3,4-tri-O-benzyl-β-D-glucopyranoside (**16**). —Glycosylation of **15** (112 mg, 0.2 mmol) with **12** (164 mg, 0.25 mmol) gave a crude product (250 mg) that was purified by chromatography (solvent *F*) to give unreacted starting alcohol (**15**, 40 mg 36%, R_f 0.87 in solvent *C*) and **16** (120 mg, 51%) as a syrup; R_f 0.32 (solvent *C*); $[\alpha]_D$ +10.5° (*c* 1, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 7.35–7.26 (m, 15 H, aromatic), 4.79 (d, 1 H, $J_{1',2'}$ 1.4 Hz, H-1'), 4.41 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 2.08–1.99 (cluster of s, 18 H, 6 × OAc), 1.92 (s, 3 H, $COCH_3$), and 0.87 (t, 3 H, *J* 6.5 Hz, CH_3); ^{13}C NMR ($CDCl_3$): δ 170.7–169.3 (7 × CO), 138.42, 138.40, 138.1 (quat. aromatic), 103.9 (C-1), 98.7, 97.9 (C-1', C-1''), 75.7, 74.9, 74.8 (CH_2Ph), 55.6 (C-2''), 23.2 ($COCH_3$), 20.7–20.6 (6 × OAc), and 14.1 (CH_3). Anal. Calcd for $C_{61}H_{81}NO_{22}$: C, 62.08; H, 6.92; N, 1.19. Found: C, 62.01; H, 6.90; N, 1.27

Octyl 2-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-α-D-mannopyranoside (**5**). —Compound **13** (81 mg, 0.108 mmol) in 0.02 M methanolic NaOMe (2.0 mL) was

kept at room temperature for 16 h. The solution was neutralized with Amberlite IRC-50 (H^+) resin, the resin was removed, and the solvent was evaporated to give **5** (51 mg, 95%) as a chromatographically homogeneous white solid; R_f 0.39 (solvent *G*). ^{1}H NMR (CD_3OD): δ 4.77 (d, 1 H, $J_{1,2}$ 1.4 Hz, H-1), 4.40 (d, 1 H, $J_{1',2'}$ 8.3 Hz, H-1'), 1.98 (s, 3 H, $COCH_3$), and 0.90 (t, 3 H, *J* 6.5 Hz, CH_3); ^{13}C NMR (CD_3OD): δ 174.3 ($COCH_3$), 101.6, 98.72 (C-1, 1'), 79.0, 78.2, 75.4, 74.9, 72.0, 71.8, 69.1 (C-2, 3, 4, 5, 3', 4', 5'), 68.7 (OCH_2CH_2), 63.3, 62.6 (C-6, 6'), 57.3 (C-2'), 23.3 (COCH3), and 14.4 (CH_2CH_3); FABMS: 518.2 $(M + Na)^+$.

*Octyl 6-O-[2-O-(2-acetamido-2-deoxy-β-*D*-glucopyranosyl)-α-*D*-mannopyranosyl]-2,3,4-tri-O-methyl-β-*D*-glucopyranoside* (**6**).—Compound **14** (120 mg, 0.126 mmol) was O-deacetylated as described for the preparation of **5**. The product was obtained as a chromatographically homogenous white powder (85 mg, 96%); R_f 0.57 (solvent *G*). ^{1}H NMR (CD_3OD): δ 4.83 (d, 1 H, $J_{1',2'}$ 1.7 Hz, H-1'), 4.45 (d, 1 H, $J_{1'',2''}$ 8.3 Hz, H-1''), 4.26 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 3.98 (dd, 1 H, $J_{2',3'}$ 2.3 Hz, H-2'), 3.53, 3.54, 3.59 (each s, 3 H, OCH_3), 1.99 ($COCH_3$), and 0.90 (t, 3 H, *J* 6.5 Hz, CH_3); ^{13}C NMR (CD_3OD): δ 174.3 ($COCH_3$), 104.6, 101.5, 99.3 (C-1, 1', 1''), 71.0 (OCH_2CH_2), 67.5 (C-6), 63.3, 62.6 (C-6', 6''), 61.0, 60.9, 60.8 (OCH_3), 57.2 (C-2''), 23.2 (COCH3), and 14.4 (CH_2CH_3); FABMS: 722.2 $(M + Na)^+$.

*Octyl 6-O-[2-O-(2-acetamido-2-deoxy-β-*D*-glucopyranosyl)-α-*D*-mannopyranosyl]-2,3,4-tri-O-benzyl-β-*D*-glucopyranoside* (**7**).—Compound **16** (118 mg, 0.10 mmol) was *O*-deacetylated as described for the preparation of **5**. The product was obtained as a chromatographically homogenous white powder (88 mg, 95%): R_f 0.72 (solvent *G*). ^{1}H NMR (CD_3OD): δ 7.32–7.20 (15 H, aromatic), 4.86 (d, 1 H, $J_{1',2'}$ 1.7 Hz, H-1'), 4.98, 4.94, 4.82, 4.74, 4.69, 4.65 (each d, $J \approx 11$ Hz, $CHHPh$), 4.46 (d, 1 H, $J_{1'',2}$ 8.3 Hz, H-1''), 4.44 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 2.00 ($COCH_3$), and 0.88 (t, 3 H, *J* 6.5 Hz, CH_3); ^{13}C NMR (CD_3OD): δ 174.3 ($COCH_3$), 140.0, 139.9, 139.7 (quat. aromatic), 104.8, 101.6, 99.5 (C-1, 1', 1''), 76.5, 75.9, 75.6 (CH_2Ph), 71.2 (OCH_2CH_2), 67.6 (C-6), 63.2, 62.6 (C-6', 6''), 57.3 (C-2''), 23.3 (COCH3), and 14.4 (CH_2CH_3); FABMS: 950.0 $(M + Na)^+$.

Enzyme testing of acceptor analogues.—Partial purification and assay of hamster kidney *N*-acetylglucosaminyltransferase-V was carried out essentially as previously described[7]. The enzyme used had a specific activity of 2.3 mU/mg, where 1 mU of enzyme activity is the amount of enzyme transferring GlcNAc from UDP-GlcNAc (1.1 mM) to trisaccharide acceptor **3** (400 μM) at a rate of 1 nmol/min at 37°C in 50 mM Na-cacodylate, pH 6.5, with 20% glycerol, 10 mM EDTA and 0.1% Triton X-100, and 1 mg/mL bovine serum albumin (Buffer A). Trisaccharide analogues **5** and **6** were evaluated as acceptors at varying concentrations (150–4000 μM of **5**; 6–400 μM of **6**) by incubating with 1.2 μU GlcNAcT-V and 11 nmol UDP-GlcNAc (30 000 dpm/nmol). Acceptor analogues and radiolabelled sugar–nucleotide donor were lyophilized in 600 μL plastic microfuge tubes; enzyme in Buffer A was added to give a final volume of 10 μL. Due to solubility problems, acceptor analogue **7** was dissolved in 1:4 Me_2SO–Buffer A and added as a solution (0.12–8 nmol) to 22 nmol of lyophilized UDP-[^{3}H]GlcNAc; Me_2SO–Buffer A was added to bring

the volume to 10 μL, and then 1.4 μU enzyme in 10 μL Buffer A was added, so that the final reaction mixtures contained 10% Me_2SO in a final volume of 20 μL. For comparison, reaction rates were measured using the regular trisaccharide acceptor **3** as substrate under the same conditions, adding first 10 μL of 1:4 Me_2SO–Buffer A to lyophilized acceptor **3** (0.12–8 nmol) and donor, and then 10 μL of enzyme in Buffer A. Tubes containing substrates and enzyme were vortexed, microfuged briefly, and incubated at 37°C for 30–62 min. Reactions were quenched by addition of water (0.4 mL), and mixtures were transferred with water onto C_{18} SepPak cartridges that had been pre-equilibrated with MeOH and then water. Unreacted radiolabelled donor was removed by washing with water (100 mL), and labelled product was eluted slowly with MeOH (3 mL) and collected for liquid scintillation counting. Apparent Michaelis constants ($K_{m,app}$) were obtained by fitting rate data to the Michaelis–Menten equation[15] using unweighted nonlinear regression (SigmaPlot 4.1, MacIntosh version).

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